Doctoral thesis

Investigation of chronic and acute epileptiform activity induced by 4-aminopyridine on seizure discharges and synaptic transmission

Sándor Borbély
Ph.D student

Consultant: **Dr. Ildikó Világi**

Doctoral School: **Biology Doctoral School**
Head of Doctoral School: **Dr. Anna Erdei**,

Doctoral Program: **Neuroscience and Humanbiology**
Head of Doctoral Program: **Dr. László Détári**,

Department of Physiology and Neurobiology, Eotvos University
Budapest
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INTRODUCTION

Epilepsy is functional disorder of nervous system with several forms of manifestations. It characterized by abnormally synchronous firing of neurons, which appears in recurrent, unprovoked seizures. This phenomenon originates from the imbalance of excitatory and inhibitory processes, resulting in overactivity of neural networks. The main excitatory neurotransmission system of mammalian forebrain uses glutamate to mediate information between neurons, so it can be a key role in the induction and maintenance of overactivity.

Many epilepsy syndromes insist upon limbic- or thalamocortical system, therefore the investigation of the role of these structures in abnormal neural synchronized activity has high importance. Several information are available about neural disorders, however exact role of different types of neurons, receptors and their contribution to the excitability of networks are still not revealed or questionable.

THE AIM OF OUR STUDY

In our present experiments we induced seizure activity in freely moving animals or in brain slice preparations by 4-aminopyridine (4-AP), to investigate the role of network compartments in limbic system and somatosensory cortex for better understanding the processes which lead to prolonged recurrent seizure activity. Our main questions were:

- Has any effect of the unilateral ablation of entorhinal cortex as highly seizure susceptible cortical region on the induction of seizure activity?
- Are repeated, mild convulsive events able to modulate the seizure threshold of limbic system, including entorhinal cortex and other brain regions, like somatosensory cortex?
- What is the receptorial background of differences, if they exist?
- How the local circuits of somatosensory cortex are involved in generation and maintenance of epileptiform activity?
- How the acute seizure activity can be inhibited by antiepileptic drugs in somatosensory cortex?

METHODS

- *In vivo* EEG recordings for comparative study of hippocampal 4-AP induced generalized tonic-clonic seizures in unilaterally entorhinal cortex ablated and sham operated adult rats.
- In brain slice experiments, electrophysiological investigation of modulation in glutamatergic neurotransmission due to mild repeated 4-AP induced seizures in limbic system and somatosensory cortex.
- Histological analysis of changes in calcium permeability of non-NMDA receptors in rats got over mild repeated seizures with cobalt uptake method.
- Identification of local microcircuit elements of somatosensory cortex involved in the generation and maintenance of acute 4-AP induced seizure like events, using current source density (CSD) analysis and recording of intrinsic optical signals (IOSs).
- Investigation of pharmacological manipulation of synaptic transmission enhanced by 4-AP application in brain slice preparation.

EXPERIMENTAL RESULTS

Consequences of left lateral entorhinal cortex lesioning

The mean latency time of first generalized tonic-clonic seizure was significantly higher in entorhinal cortex ablated animals compared to sham operated controls, while the number of seizure event within the first 3h was lower. The hippocampal EEG pattern of convulsive states was also different. The duration of tonic phase of grand mal seizures increased, without changes in frequency, while a reduction in frequency of clonic phase was observed with no changes in duration. We can conclude that the seizure susceptibility of ablated animals was higher, which manifested in rise of seizure latency, shrinkage of the number of seizures and intensity.

The effect of repeated 4-AP seizures on excitatory synaptic transmission

The effect of repeated seizures elicited by 4-AP were studied in *in vitro* brain slice preparation. According to our observations the early, there were changes in AMPA receptor mediated synaptic components of field excitatory postsynaptic potentials (fEPSP) , while no

functional differences were found in late, NMDA receptor mediated components. The analysis of early component of fEPSP revealed an increase of general excitability in entorhinal cortex and hippocampal CA1 area, while mild decrease was detected in somatosensory cortex. Our pharmacological experiments showed reduced antagonistic effect of GYKI 52466 (1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine), a selective AMPA receptor antagonist in both area of limbic system, and unchanged efficacy in somatosensory cortex. Altough the NMDA receptor antagonist APV (D-2-amino-5-phosphono-valeric acid) reduced the late component of fEPSP in every case, no differences were found in its efficacy within different brain regions. To unveil the possible functional alterations of NMDA receptor dependent processes, the long-term potentiation induction was also compared in 4-AP treated and control animals. The preceding seizure events had not exerted any effect on long-term potentiation induction in any brain regions.

The cobalt uptake experiments of slices from 4-AP treated animals showed a decrease in cobalt uptake in layer 2/3 and 5 of somatosensory and layer 1-3 of entorhinal cortices, while an increase was observed in the hippocampal CA1 area and the dentate gyrus. This method is specific for non-NMDA receptors activation, when calcium ions can pass the membrane, so according to our results bivalent ion, including calcium conductance of AMPA and Kainate receptors have changed significantly.

Identification of microcircuit elements involved in the maintenance of seizure activity

Seizure like events were elicited in brain slices of rat somatosensory cortex by 20 μM 4-AP and spontaneous events were recorded continuously using a 16 channel multilelectrode system. The intrinsic optical signals were also recorded for off-line analysis.

The increase of reflected light intensity of brain tissue was observed due to convulsant application. Highest changes of slice luminance was detected in the part of primary somatosensory cortex bordering with somatomotory cortical region, in the secondary somatosensory cortex, hippocampal CA1 area, dentate gyrus and subiculum. The current source density analysis of spontaneous field potentials revealed the presence of high intensity synaptic currents in supragranular layers in conformity with intrinsic optical experiments.

Pharmacological manipulation of acute 4-AP induced epileptic condition

In the experiments performed by specific channel blocker or receptor antagonists, lamotrigine effectively reduced the early component of fEPSP both in control and 4-AP treated slices in a dose of 50 μM, while it was able reduce the amplitude of late component in

4-AP treated slices. Memantine, in 1.5μM concentration, inhibited the early component of fEPSP in control slices and reduced the late component developed following 4-AP application

DISCUSSION

The 4-AP model

The voltage-gated potassium channel blocker 4-AP is a widely used convulsant both in *in vivo* and *in vitro* experiments (Fragoso-Veloz et al., 1990). 4-AP has short range effect on neuronal excitability, after removal from tissue the enhanced activation state disappear, without long-term consequences. Due to the lack of neurodegenerative symptoms in treated animals spontaneous seizures never develop following the acute phase of treatment. This is why 4-AP is an ideal compound for eliciting overactivation in brain tissue.

The effect of unilateral entorhinal cortex ablation

The explanation for the increased seizure tolerance of entorhinal cortex lesioned animals can be the well documented participation of this region in seizure generation. Experiments on rat brain slices demonstrated that 4-AP treatment can provoke interictal activity originated from hippocampus, which prevent the development of ictal-like activity of rhinal cortices. Following surgical separation of hippocampus and entorhinal cortex ictal-like activity appears in rhinal cortices (Barbarosie and Avoli, 1997). Disinhibition of entorhinal cortex also leads to the emerging of epileptiform activity (de Curtis and Pare, 2004). Due to the wide-spread connections of entorhinal cortex with hippocampus the epileptiform discharges can easily invade all hippocampal regions and re-enter in augmented form, so the removal of this cortical region can lead to the rise of seizure threshold.

Discussion of the effects of chronic 4-AP treatment

Our collaborators at the Department of Anatomy, Faculty of Medicine, University of Szeged demonstrated the rearrangement of glutamate receptor subunit compostion due to repeated mild convulsions, using histoblot method. Changes of subunit expression were compared with our electrophysiological results, for better understand the underlying mechanisms of functional alterations.

The most important feature of nervous system is making the adaptation of an organism for varying environment possible. The capability of neural plasticity is based on

morphological and receptorial changes of synapses. The alteration of receptor number, subunit composition or phosphorilation state has high importance, and determines the neural transmission, and its effect on postsynaptic neuron.

Our experiments made on brain slices of rats got over repeated 4-AP treatment demonstrated the increase of AMPA receptor mediated processes, while NMDA receptor dependent transmission remained unchanged.

Previous experiments revealed that NMDA receptors are concerned in the epileptic processes, especially in the maintenance of overactivation. Since no difference in the APV efficacy was found, we can assume that in our model no changes occurred in the NMDA receptor mediated synaptic processes. This hypothesis is supported by our investigations made on eliciting long-term potentiation. The 4-AP treatment had no effect on the long-term potentiation, which process depends on the prolonged activation of NMDA receptors inducing enhancement of synaptic transmission. According to our histoblot results the NR2A subtype of NMDA receptors was upregulated, while the expression of other subtypes was unchanged. Convulsive events induce the downregulation of NR2A subunit in mature nervous system, resulting in enhanced seizure responsiveness (Silva et al., 2005; Zhu et al., 2004). This observation is in agreement with our results, the expression of subunit changed in opposite direction, while a seizure tolerance developed in treated animals. Since the NR2A subunit containing NMDA receptors are characterized by lower ionic current and inactivation time constant, the overexpression of this subtype resulting in a decreased current flow through the NMDA receptors (Cull-Candy és mtsai, 2001).

Our electrophysiological results demonstrated the enhancement of AMPA receptor mediated synaptic currents in limbic system while it remained unchanged in somatosensory cortex. Significant alterations were found in the calcium permeability of non-NMDA receptors. According to the cobalt uptake measurements the shrinkage of calcium permeability was detected in the somatosensory and entorhinal cortices, while an increase was observed in the hippoacampal CA1 area and dentate gyrus. The calcium permeabilltiy of AMPA receptors depends on the subunit composition, in the presence of GluR2 subunit the receptor is impermeable to calcium ions (Bigge, 1999). Due to the different subunit composition of kainate receptors, these are permeable to calcium ions. Neurodegenerative insults usually induce the downregulation of GluR2 subunit in the mostly affected brain region, resulting in a prolonged elevation of intracellular calcium concentration and finally cell death (Pellegrini-Giampietro et al., 1997). Our histoblot results demonstrated the mild downregulation of GluR2 subunit in limbic system, which can explain the increased calcium

permeability demonstrated in hippocampal regions. The calcium permeability decrease observed in the entorhinal cortex might occur due to the reduced KA-2 subunit expression, which compensated the GluR2 downregulation. Since our electrophysiological results established the same functional alteration within the limbic system the similar expression change of GluR2 subunit might be the key factor in determining the strength of synaptic transmission. The lack of GluR2 expression change in the somatosensory cortex resulted in that the synaptic efficacy remained unchanged due to the 4-AP pretreatment. Contradictory the calcium permeability decreased in the supragranular layers which might occur due to the downregulation GluR1-4 and KA-2 subunits.

The 4-AP treatment resulted in the rise of seizure threshold, supposing that in the other not investigated brain regions weakening of synaptic currents induced in a similar way, demonstrated in the somatosensory cortex.

Identification of microcircuit elements involved in the maintenance of seizure activity

Our method based on the measurement of intrinsic optical changes and the analysis of current source density revealed the strong activation of supragranular layers both in the primary and secondary somatosensory cortex. Numerous study documents the involvement of layer 5 intrinsically bursting neurons in seizure activity in rodent epilepsy models (Chagnac-Amitai and Connors, 1989). The possible explanation of our contradictory results can be the higher activity of layer 2/3 neurons due to the preparation method. Damage of brain tissue during surgery is more harmful for interneurons than principal cells, resulting in higher rate of cell death in inhibitory neurons. Probable the seizure focus can be localized within layer 2/3 without selective loss of interneurons as it is present in human epileptic tissue preparation (Köhling et al., 1999).

Pharmacological manipulation of acute 4-AP induced epileptic condition

Two antiepileptic drugs were used in our pharmacological experiments with different inhibitory mechanisms. The common feature of their effect is the lack of interaction with potassium channels.

Lamotrigine, as a voltage-gated sodium channel antagonist reduce the excitability of neurons and therefore inhibits the appearance of seizures. In our acute model lamotrigine exhibited higher antagonist efficacy in 4-AP treated slices than in controls, therefore other inhibitory mechanisms are supposed. The direct effect of lamotrigine on glutamatergic receptors are not known, therefore other process affecting synaptic transmission must be

assumed. Experiments performed on rat amygdala proved the binding of lamotrigine to voltage-gated calcium channels resulting in a decrease in GABAergic transmission (Braga et al., 2002). 4-AP enhances the synaptic transmission by the activation of presynaptic calcium channels, so this antagonistic mechanism is real possibility.

Memantine is widely used non-competitive NMDA receptor antagonist, however in higher dose it can bind to almost every modulatory neurotransmission system (Parsons et al., 1999). Since the late component of fEPSP is mediated by the NMDA receptors the reduced amplitude of this constituent was expected. Memantine effectively inhibited the late component in 4-AP treated slices, while it had no effect in the lack of convulsant. The reason might be that, during different pathological conditions accompanying with prolonged increased extracellular glutamate concentration memantine exhibits higher efficacy (Chen and Lipton, 1997). This hypothesis is supported by our observation, whereas the inhibitory potential was independent of stimulus intensity, so presynaptic release has no effect.

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